example, on page 24, line 33 - page 25, line 31. In addition, applicants have amended claim 1 to recite that the crosslinked protein crystal is capable of "controlled dissolution" from an insoluble form to a soluble form. Support for this amendment can be found in the specification, for example, on page 7, line 22 - page 8, line 8.

Applicants have amended claim 39 to recite that the protein delivery system comprises a delivery device in addition to the crosslinked protein crystals. This amendment is supported in the specification, for example, on page 20, line 16 - page 21, line 12.

Applicants have canceled claim 80, without prejudice.

None of these amendments constitutes new matter.

35 U.S.C. § 112, first paragraph

Claims 1-60, 76-77, and 80-85 stand rejected under 35 U.S.C. § 112, first paragraph, on the basis that "[t]he specification, while being enabling for crosslinking with a multifunctional crosslinking agent, does not reasonably provide enablement for other crosslinking agents." Applicants traverse, based on the claim amendments and arguments presented herein.

Applicants have amended claims 1, 17, 18, 54-56 and the claims which depend therefrom, to recite that the crosslinker is a multifunctional crosslinking agent, thus obviating the rejection.

For this reason, the rejection under 35 U.S.C. § 112, first paragraph should be withdrawn.

35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 30, 31, 39-44, 67-75 and 80 under 35 U.S.C. § 112, second paragraph "for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

Claims 30 and 40 stand rejected on the basis that the meaning and scope of "decontamination proteins" is uncertain. Applicants traverse. The use of proteins for decontamination is referred to throughout the specification, for example, on page 20, lines 23-24.

Claim 31 stands rejected on the basis that "it is uncertain as to vitamins that are a protein." Applicants have obviated this rejection by amending claim 31 to delete this term.

Claims 39-44 stand rejected on the basis that it is "unclear as to how they further limit claims 1, 17 or 18 by requiring a delivery system since the system is required to contain only the crossliked protein of claims 1, 17 or 18." Applicants have obviated this rejection by amending claims 39-44 to recite that the intended protein delivery systems additionally comprise a delivery device.

Claims 67-75 stand rejected on the basis that the substance that contains the percentages of crosslinking agent and

the basis of the percentages are unclear. Applicants have amended claims 67-75 to recite that the percentages are based on the composition of the slurry which contains the crosslinked protein crystals.

Claim 80 stands rejected as being redundant in view of claim 76. Applicants have obviated this rejection by canceling claim 80.

Applicants request that the Examiner reconsider and withdraw all the outstanding rejections under 35 U.S.C. § 112, second paragraph.

35 U.S.C. § 102(a)

Claims 1-44, 46-63, 76 and 90-95 stand rejected under 35 U.S.C. § 102(a) on the basis that they are "anticipated by" Navia et al. (United States patent 5,618,710) ("Navia"). Specifically, the Examiner contends that "Navia et al disclose crosslinked protein crystals that are inherently capable of being changed form insoluble to soluble form by one or more of the changes claimed. The present claims encompass crosslinked protein crystals and methods for preparation thereof disclosed by Navia et al." Applicants traverse.

As an initial matter, applicants point out that the crystals of Navia are not inherently capable of change to soluble form by one or more of the presently indicated triggers. In fact, just the opposite is true. The crystals of Navia are intended to

be refractory to the triggers recited in the present claims. In particular, Navia is directed to crosslinked protein crystals that are crosslinked to such an extent that the resulting crystals can "function at elevated temperatures, extremes of pH, and in harsh aqueous, organic, or near-anhydrous media" (column 3, lines 49-51). Furthermore, the crosslinked enzyme crystals of Navia are intact crystal catalysts which "can function in environments incompatible with the functional integrity of the corresponding, uncrystallized, uncrosslinked, native enzyme or conventionally immobilized enzyme catalysts" (column 3, lines 53-56). The crystals of Navia are clearly not intended to "change" into soluble form, under even extreme conditions.

In purported support for the contention that the crystals of Navia and applicants' invention must be the same, the Examiner cites Example 4 of Navia, wherein the concentration of glutaraldehyde used was 5.77% and Example 9 of Navia, wherein 2% glutaraldehyde was used. The Examiner concluded that "if the crosslinking conditions can be the same or essentially the same, the presently claimed crosslinked crystals must be the same or essentially the same."

In the disclosure of the instant invention, applicants have identified a number of factors which are relevant to the release rate of protein activity of crosslinked protein crystals (see, for example, page 12, lines 12-24). Importantly, the type and amount of crosslinker are just two of the relevant factors.

Upon closer inspection, the many differences between the instant invention and the disclosure of Navia overwhelm any noted similarities.

For example, Navia's Example 9 describes the crosslinking of Jack Bean urease using 2% glutaraldehyde. The closest examples in the instant specification (as pointed out by the Examiner in terms of percent crosslinker used) would be Examples 18-23, where applicants crosslink Candida rugosa lipase with glutaraldehyde at percentages ranging from 4% to 6.5%. As set forth in the present specification, applicants have clearly disclosed that the type of protein being crosslinked is vital in determining the conditions to be used to produce a protein crystal capable of controlled dissolution. For example, the amino acid residues involved in the crosslinks, the surface area of the crosslinked protein crystal, and the size of the crosslinked protein crystal are all important in determining whether the amount of crosslinker used will result in a protein crystal capable of controlled dissolution (as in the instant invention) or a protein crystal effectively locked in place by crosslinking (as in Navia). It is therefore extremely important that the Examiner, when evaluating the relevance of Navia, consider the many differences between Jack Bean urease and Candida rugosa lipase. Some of these differences are summarized in the following chart.

Enzyme Aspect	Candida rugosa lipase	Jack Bean Urease	Pancreatic Elastase
Mw	59kD	550kD	28.7kD
Subunits	1	6	1
Carbohydrate	7%	0	0
pI	5.08	6.05	10.49
Total Lysines	3.1%	6.05%	.4%
average hydropathicity	-0.014	-0.152	-0.012

As can be seen, there are significant differences between these three proteins that can dramatically affect crosslinking: for example, comparing Candida rugosa lipase and Jack Bean urease, the lipase is much smaller than the urease, contains fewer lysine residues (lysine is the primary crosslinking residue) and Candida rugosa lipase is composed of only one subunit, as compared to six in the urease. Comparing the lipase to Pancreatic elastase, although the proteins are similar in size and have the same number of subunits, the lipase has a much higher carbohydrate content. Carbohydrates interfere with the ability of the crosslinker to access the protein and result in a less crosslinked protein compared to a similar protein with less carbohydrates. In other words, if two proteins were subjected to identical crosslinking conditions, the protein with the higher carbohydrate content would not become as extensively crosslinked as the protein with the lower carbohydrate content. Therefore, contrary to the Examiner's assertion, treatment of these three different proteins with similar levels of glutaraldehyde would be predicted to result in

quite different levels of crosslinking. In other words, the mere fact that the glutaraldehyde conditions of the instant invention may appear to overlap with those described by Navia is meaningless, when the crystals being crosslinked are completely different.

Based on these significant differences between the instant invention and Navia, applicants submit that the crosslinking conditions are not "essentially the same" and therefore would not result in crosslinked crystals that "must be the same or essentially the same". Applicants request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 102(a).

35 U.S.C. § 103(a)

Claims 45 and 64-75 stand rejected under 35 U.S.C. § 103(a) as "being unpatentable over" Navia. Specifically, the Examiner contends that it "would have obvious to use a crosslinked enzyme crystal such as a protease produced as disclosed by Navia et al in a detergent formulation required by claim 45 since it is conventional to use enzymes such as proteases in detergent formulations...."

As described above, Navia does not anticipate the controlled dissolution crystals of the instant invention. Further, nothing in Navia suggests such crystals. In fact, Navia teaches away from controlled dissolution crystals by focusing on

the inherent stability of Navia's crosslinked crystals and their ability to withstand harsh conditions. In so doing, Navia teaches away from experimenting with variables such as time, percentages of crosslinkers and combinations of crosslinkers in methods to produce controlled dissolution crystals. Therefore, the controlled dissolution crystals of the instant invention and the methods for producing them would not have been obvious based on Navia.

Claims 1-62 and 76-85 stand rejected as being unpatentable over Navia, in view of Kausch et al. (United States patent 5,508,164), and if necessary in further view of Neville et al. (United States patent 5,066,490). Applicants traverse.

As described above, Navia does not teach or suggest the controlled dissolution crosslinked protein crystals of the instant invention. And neither Kausch et al. nor Neville et al. make up the deficiencies in Navia in that respect.

Applicants therefore request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 103(a).

CONCLUSION

Applicants request that the Examiner consider the foregoing amendments and remarks and pass this application to issue.

Respectfully submitted,

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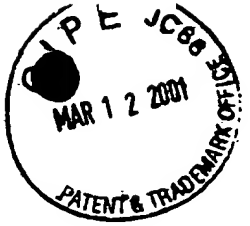

Appendix A

1. (Amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent and said protein crystal being capable of [change] controlled dissolution from insoluble and stable form to soluble and active form upon a change in the environment surrounding said crystal, said change being selected from the group consisting of: change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystal and combinations thereof.

17. (Amended) A crosslinked protein crystal, said protein crystal being crosslinked with a multifunctional crosslinking agent and said protein crystal having a half-life of activity under storage conditions which is greater than at least 2 times that of the soluble form of the protein that is crystallized to form said crystal that is crosslinked and activity similar to that of the soluble form of the protein under conditions of use.

18. (Amended) A crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent and said protein crystal being capable of releasing its protein activity at a controlled rate upon exposure to a change in the environment surrounding said crystal, said change being selected from the group consisting of change in pH, change in solute concentration, change in temperature, change in chemical composition, change in shear force acting upon the crystals and combinations thereof.

31. (Amended) The crosslinked protein crystal according to any one of claims 1, 17 or 18, wherein said protein is selected from the group consisting of hormones, antibodies, inhibitors, growth factors, trophic factors, cytokines, lymphokines, toxoids, growth hormones, nerve growth hormones, bone morphogenic proteins, toxoids[, vitamins] and nutrients.

39. (Amended) A protein delivery system, said system comprising crosslinked protein crystals according to any one of claims 1, 17 or 18 and a delivery device.

48. (Amended) A pharmaceutical controlled release formulation comprising a crosslinked protein crystal, said protein crystal being crosslinked by a multifunctional crosslinking agent and said crystal being substantially insoluble under storage conditions and capable of releasing its protein activity [in vivo] *in vivo* at a controlled rate.

54. (Amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are characterized by the ability to change from insoluble and stable form to soluble and active form upon a change in their environment, said change being selected from the group consisting of change in temperature, change in pH, change in chemical composition, change from concentrate to dilute form, change in shear force acting upon the crystals and combinations thereof.

55. (Amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are characterized by a half-life of activity under storage conditions which is greater than at least 2 times that of the soluble form of the protein that is crystallized to form said crystals that are crosslinked and activity similar to that of the soluble form of the protein under conditions of use.

56. (Amended) A method for producing crosslinked protein crystals comprising the step of reacting protein crystals in a slurry with a first multifunctional crosslinking agent, or a first multifunctional crosslinking agent and at least a second multifunctional crosslinking agent, under conditions sufficient to induce crosslinking of said crystals to the extent that the resulting crosslinked crystals are characterized by being capable of releasing their protein activity at a controlled rate upon exposure to a change in their environment, said change being selected from the group consisting of change in pH, change in soluble concentration, change in temperature, change in chemical composition, change in shear force acting upon the crystals and combinations thereof.

67. (Amended) The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is [0.0076% to 0.5%] glutaraldehyde at a concentration of 0.0076% to 0.5% in the slurry and wherein the conditions sufficient to induce

crosslinking include reacting protein crystals with a crosslinking agent for a period of time between about 3 minutes and about 120 minutes.

68. (Amended) The method for producing crosslinked protein crystals according to claim 67, wherein said crosslinking agent is [0.005%] glutaraldehyde at a concentration of 0.005% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with a crosslinking agent for a period of time between about 10 minutes and about 30 minutes.

70. (Amended) The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is [0.01% to 1%] glyoxal at a concentration of 0.01% to 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with a crosslinking agent for a period of time between about 30 minutes and about 60 minutes.

71. (Amended) The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is [0.05% to 1%] octanedialdehyde at a concentration of 0.05% to 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with a crosslinking agent for a period of time between about 30 minutes and about 16 hours.

72. (Amended) The method for producing crosslinked protein crystals according to claim 71, wherein said crosslinking agent is [1%] octanedialdehyde at a concentration of 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with a

crosslinking agent for a period of time between about 1 hour and about 3 hours.

73. (Amended) The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said crosslinking agent is [1%] succinaldehyde at a concentration of 1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting protein crystals with a crosslinking agent for a period of time between about 30 minutes and about 3 hours.

74. (Amended) The method for producing crosslinked protein crystals according to any one of claims 54, 55 or 56, wherein said first crosslinking agent is [0.01% to 4%] epoxide at a concentration of 0.01% to 4% in the slurry and said second crosslinking agent is [0.1% to 0.2%] glutaraldehyde at a concentration of 0.1% to 0.2% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting said protein crystals with said first crosslinking agent for a period of time between about 1 hour and about 72 hours and reacting said protein crystals with said second crosslinking agent for a period of time between about 1 hour and about 5 hours.

75. (Amended) The method for producing crosslinked protein crystals according to claim 74, wherein said first crosslinking agent is [0.01%] epoxide at a concentration of 0.01% in the slurry and said second crosslinking agent is [0.1%] glutaraldehyde at a concentration of 0.1% in the slurry and wherein the conditions sufficient to induce crosslinking include reacting said protein crystals with said first crosslinking agent for about 5 hours and reacting said protein crystals with said second crosslinking agent for about 1.5 hours.

Please cancel claim 80.

81. (Amended) The method for producing crosslinked protein crystals according to <u>any one of</u> claim<u>s</u> [80] <u>54, 55 or 56</u>, wherein said enzyme is selected from the group consisting of hydrolases, isomerases, lyases, ligases, transferases and oxidoreductases.